

A Scalable Synthesis of MN-447, an Antagonist for Integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$

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Abstract:

(2*S*)-Benzenesulfonylamino-3-[3-methoxy-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid, MN-447, is a potent antagonist of the integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$. Herein, we report a novel synthetic protocol that produces MN-447 in an overall yield of 45%. This protocol, when compared with the original synthetic route for MN-447, is more cost-effective, requires fewer steps, does not require chromatographic purification of intermediates and MN-447, and increases the overall yield by 35%. This report focuses on the synthetic strategies that were developed for this protocol. Now, the large quantities of MN-447 that are needed for preclinical and toxicological studies can be readily obtained.

Introduction

The vitronectin receptor, integrin $\alpha_v\beta_3$, mediates the adhesion and migration of vascular smooth muscle cells and leukocytes.¹ Another member of the integrin family, integrin $\alpha_{IIb}\beta_3$, functions at the convergence point for the various pathways that lead to platelet aggregation because, as a platelet surface receptor, it binds fibrinogen.² Antagonists that block integrin $\alpha_{IIb}\beta_3$ –fibrinogen binding prevent platelet aggregation; therefore, integrin $\alpha_{IIb}\beta_3$ antagonists^{3,4} are used to treat coronary thrombosis. The Fab fragment of a human-murine monoclonal antibody, Abciximab,⁵ which binds to both integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$, is used to treat ischemic diseases. Small, nonproteinaceous, water-soluble molecules that are inexpensive to synthesize and act as antagonists of integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$ would likely be of therapeutic value in the treatment of acute ischemic diseases. During the course of our efforts to develop such antagonists,^{6–9} we discovered (2*S*)-benzenesulfonylamino-3-[3-

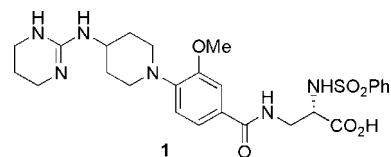


Figure 1. MN-447 / CP4715 (1).

methoxy-4-{4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl}benzoylamino]propionic acid (1), named at the time, CP4715 (Figure 1).⁷ The compound is a strong antagonist of both integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$. Its water solubility and preliminary ADMET profiles suggest that it might be an infusible drug candidate. In a canine ischemia–reperfusion model, 1 showed superior cardioprotective efficacy, e.g., reduction in infarct size after reperfusion, and also showed a low risk of bleeding.¹⁰ In 2006, 1 was licensed to Medicinova, Inc., which is a development-stage biopharmaceutical company focusing on acquiring, developing, and commercializing high-quality small-molecule therapeutics.¹¹ CP4715, renamed MN-447, is currently in preclinical development.

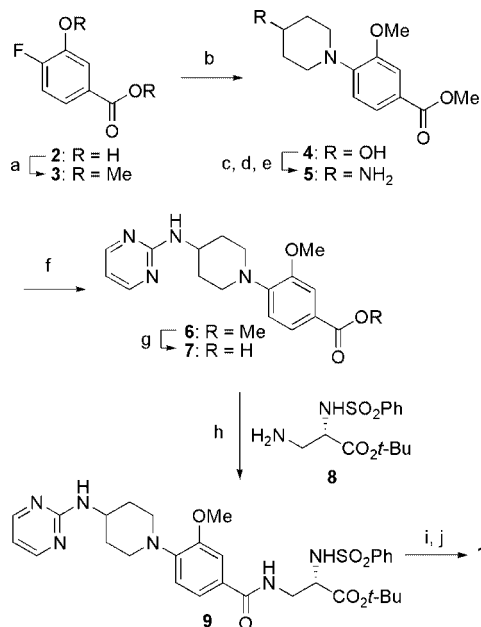
The original medicinal chemistry-based route⁷ of 1 is outlined in Scheme 1. Commercially available fluorobenzoic acid 2 is simultaneously methylated at the hydroxyl and carboxyl groups to give the 4-fluorobenzoate derivative 3. Nucleophilic substitution of 3 with 4-hydroxypiperidine gives the bicyclic alcohol 4 that is then converted after three steps—one of which involves reaction with sodium azide and produces an azide intermediate—to the amine 5. Introduction of a pyrimidine moiety gives 6. Base hydrolysis of 6 gives the carboxylic acid 7, that is then coupled with the diaminopropionate 8¹² to afford the amide 9. Finally, removal of the *tert*-butyl group with TFA followed by hydrogenation of the pyrimidine ring gives the desired molecule 1 as a noncrystalline solid. The synthesis outlined above is ill-suited for large-scale production of 1. The overall yield of 1 (10.5% yield¹³) and the need for expensive chemicals prohibitively increase the production cost. The products of steps a, b, f, h, and j must be purified chromatographically. Additionally, hazardous azides are involved. As an alternative, we have developed and report

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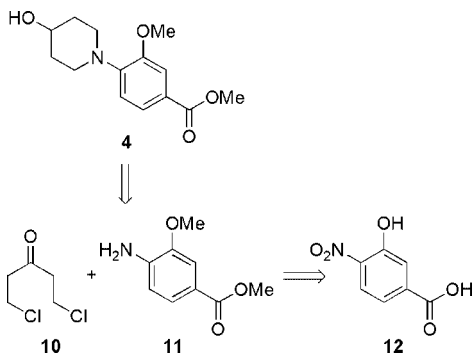
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- (13) The yields are uncorrected for purity.

Scheme 1. Medicinal chemistry route of 1^a



^a Reagents: (a) MeI, K₂CO₃, DMF, rt, 17 h, chromatography, 82%;¹³ (b) 4-hydroxypiperidine, DMSO, 90 °C, 13 h, chromatography, 47%;¹³ (c) MsCl, Et₃N, CH₂Cl₂ rt, 15 min; (d) NaN₃, DMF, 90 °C, 4.5 h; (e) Pd/C, H₂, 1,4-dioxane, rt, 6 h; (f) 2-bromopyrimidine, *i*-Pr₂EtN, DMSO, 120 °C, 15 h, chromatography, 64%¹³ in 4 steps; (g) NaOH, THF, MeOH, H₂O, 40 °C, 5 h, 68%¹³; (h) EDC, HOBT, *N*-methylmorpholine, DMF, rt, 12 h, chromatography, 100%;¹³ (i) TFA, CH₂Cl₂, rt, 16 h; (j) 10% Pd/C, H₂, 1,4-dioxane, H₂O, rt, 5 h, chromatography, 63%¹³ in 2 steps.

Scheme 2. Retrosynthesis of 4



herein a more efficient means for the synthesis of **1** that avoids the aforementioned shortcomings of the original protocol.

Results and Discussion

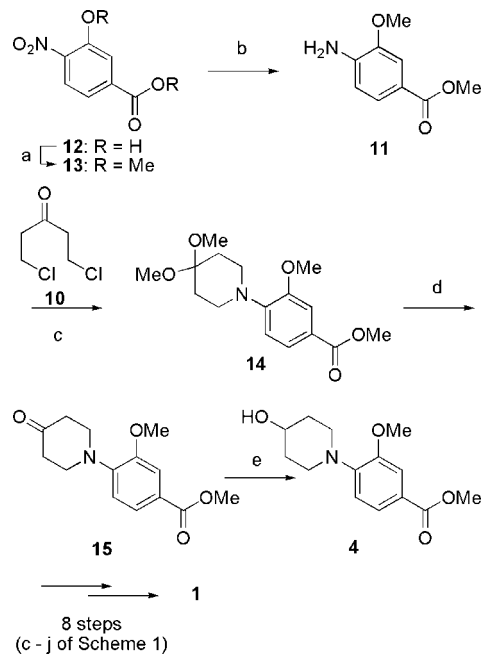
We first developed an alternative route for the synthesis of **4** that circumvents the nucleophilic substitution reaction, thereby eliminating the expensive compound **2** (14,800 yen/g), and the chromatographic separation of the complex product mixture. A retrosynthesis of **4** is outlined in Scheme 2. Reactions of anilines with 1,5-dichloro-3-pentanone¹⁴ (**10**) produce piperidine rings.^{15,16} We thought that such a reaction might be a suitable step during the large-scale production of **1** because, generally, anilines (e.g., **11**) and their precursor nitro-derivatives (e.g., **12**)

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Scheme 3. Second generation route for 1^a



^a Reagents: (a) MeI, K₂CO₃, DMF, rt, 95%; (b) 10% Pd/C, H₂, 1,4-dioxane, MeOH, H₂O, 35 °C, 96%; (c) TsOH H₂O, MeOH 60°C; (d) HCO₂H, H₂O, rt, 2 h, chromatography, 58%¹³ in 2 steps; (e) NaBH₄, MeOH, rt, chromatography, 95%.¹³

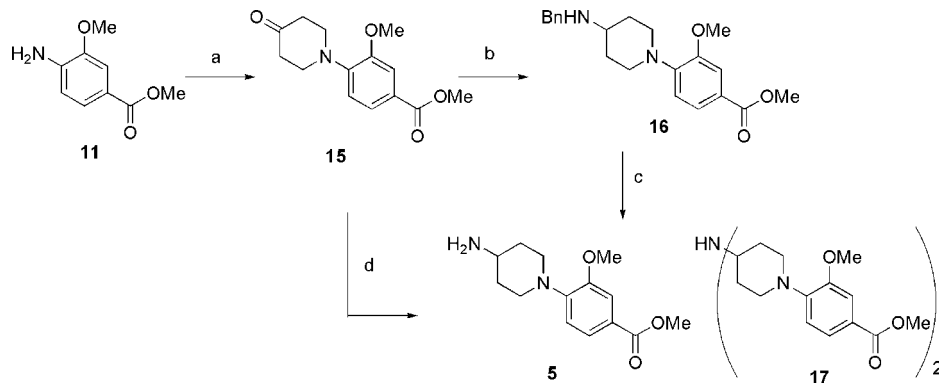
are less expensive than are the corresponding fluoro derivatives. Therefore, the inexpensive 3-hydroxy-4-nitrobenzoic acid (**12**) (289 yen/g) was selected as a starting material.

A second generation route for the synthesis of **1**, is shown in Scheme 3. Introduction of two methyl groups into **12** gave methyl 3-methoxy-4-nitrobenzoate (**13**) as a pale yellow solid in 95% yield at 98.3% purity. Hydrogenation of **13** gave the aniline **11** as a pale yellow solid in 96% yield at 100% purity. The reaction time for the hydrogenation was shortened from 20 h to 8–12 h by increasing the reaction temperature from room temperature to 35 °C. We isolated **13** and **11** as solids, without chromatographic purification, by simply pouring the product solutions into water and filtering.

We then used the piperidine ring-closure method to synthesize the ketone **15**. By following this method,¹⁵ reaction of **11** with **10** and Na₂CO₃ gave **15** in 25% yield.¹³ While attempting to improve the yield, we identified another condition that incorporated TsOH¹⁶ gave the acetal **14**. The cleavage of the acetal¹⁶ afforded **15** in 58%¹³ (two steps). Reducing the carbonyl group of **15** with NaBH₄ gave **4**, which is the product of the second step of the original route. While further investigating the piperidine ring-closure, we found that the addition of TsOH was unnecessary and that during the workup **14** converted spontaneously to **15** when the water solution containing **14** was concentrated. Consequently, the workup of **14**—addition of water, filtration, partial concentration by evaporation, neutralization, and filtration—produced **15** directly. Thus, starting with 0.95 kg of **12**, 1 kg of **15** was synthesized in 76.6% yield at 97.2% purity and isolated without any chromatographic purifications (Scheme 4).

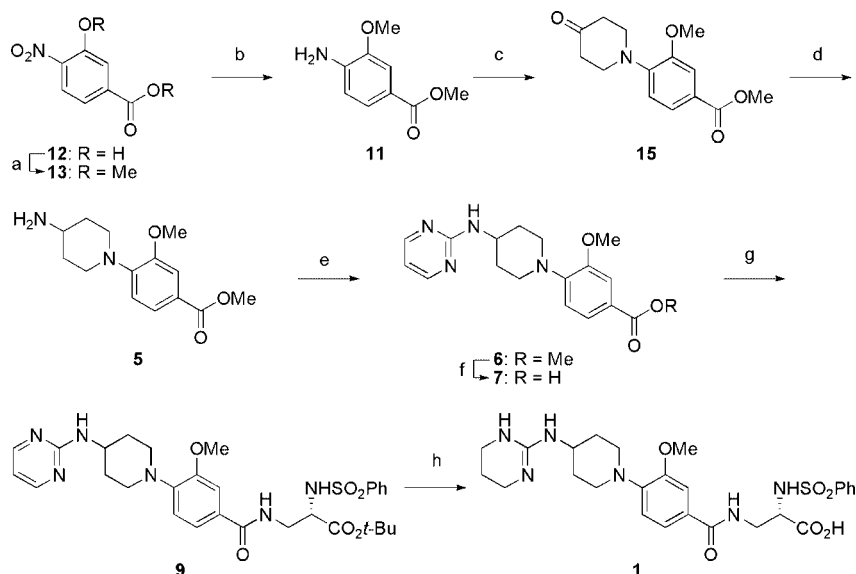
Subsequent attempts to decrease the number of steps required to synthesize **1** focused on the synthesis of **5** from **15**. We first tried a reductive amination of **15** with benzylamine. The reaction

Scheme 4. Practical route for 5^a



^a Reagents: (a) **10**, MeOH, 60 °C, 84%; (b) BnNH₂, NaBH(OAc)₃, MeOH, 4 °C, 97%;¹³ (c) 10% Pd/C, H₂, MeOH, rt, 100%;¹³ (d) 10% Pd/C, H₂, conc. NH₄OH, 1,4-dioxane, H₂O, rt, 90%.

Scheme 5. Optimized synthetic procedure for scalable synthesis of 1^a



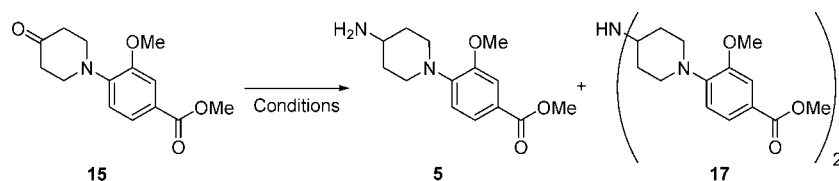
^a Reagents: (a) MeI, K₂CO₃, DMF, rt, 22 h, 95%; (b) 10% Pd/C, H₂, 1,4-dioxane, MeOH, H₂O, 35 °C, 12 h, 96%; (c) **10**, MeOH, 60 °C, 4 h, 84%; (d) 10% Pd/C, H₂, conc. NH₄OH, 1,4-dioxane, H₂O, rt, 12 h, 90%; (e) 2-chloropyrimidine, NaHCO₃, *n*-BuOH, 100 °C, 27 h; (f) NaOH, MeOH, H₂O, 50 °C, 2 h, 80% in 2 steps; (g) **8**, BOP, HOBT, *N*-methylmorpholine, DMF, 4 °C, 23 h, 96%; (h) i) 0.5 M HCl, 60 °C, 5.5 h, ii) 10% Pd/C, H₂, 0.25 M HCl, 30 °C, 7.5 h, 84%.

proceeded smoothly to give **16**. The benzyl group was removed by hydrogenation to give **5**. Thus, **5** was synthesized from **15** in two steps and in 97% yield.¹³ Additionally, the number of steps required to synthesize **5** from **15** was reduced by two.

We also considered that an even shorter route might be possible if **15** could be converted to **5** directly by a reductive amination. Therefore, reactions using various ammonia sources (NH₄OAc, NH₄CO₂H, (NH₄)₂SO₄, (NH₄)₂CO₃, NH₂OH hydrochloride, or NH₄OH) and various reducing reagents (NaBH(OAc)₃, NaBH₃CN, NaBH₄, or Pd/C under H₂) were examined. The amination using NH₄OH and Pd/C under H₂ gave the most promising results. While the reaction did not produce **4** as a byproduct, it did produce the contaminating secondary amine **17**, which resulted when **5** further reacted with **15**. We tried to reduce the amount of **17** produced by modifying the reaction conditions. Table 1 summarizes the findings of this investigation. The relative amounts (reported as percentages) of **5** and **17** reported in Table 1 were determined after measuring the areas under their respective HPLC peaks. With MeOH, as

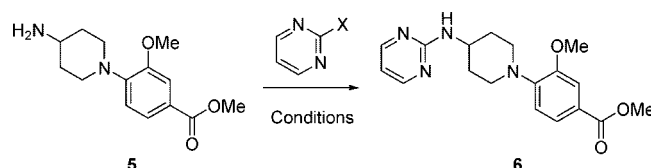
the solvent, 23% of the product mixture was **17** (entry 1). A solution containing equal volumes of 1,4-dioxane and MeOH was next tried because **15** was not completely soluble in MeOH. With 1,4-dioxane and MeOH present, the reaction produced a smaller amount of **17** (15%; entry 2). Increasing the pressure had no effect (entry 4), but decreasing the concentration of **15** reduced the amount of **17** produced (compare entries 5 and 6). Finally, the amount of **17** decreased to 8% of the product mixture when 1,4-dioxane was the solvent (entry 8). Compound **17** could be selectively removed from the product mixture by adjusting the pH of the aqueous solution to 7. In this pH, **17** was precipitated, whereas **5** was soluble in the aqueous solution. After extracting the precipitated **17** with AcO*n*-Bu, **5** was extracted from the aqueous solution (pH 10) with AcOEt. The yield of **5** was 90%, and its assay purity was 99.4%.

Having established a practical synthesis for **5**, we focused on optimizing the conversion of **5** to **1** via the intermediates of Scheme 1. First, we attempted to minimize the cost of the synthesis of **6** from **5**, which originally added a pyrimidine

Table 1. Preparation of **5** by reductive amination of **15**

entry	solvents ^a	conc. of 15 (M)	Pd/C/ 15 (wt %)	aqueous NH ₄ OH (equiv)	time (h)	5 ^b (%)	17 ^b (%)
1	MeOH	0.11	30	6	3	70.0	23.0
2	MeOH/dioxane (1/1)	0.22	30	8	4	80.0	15.2
3	MeOH/dioxane (1/1)	0.22	10	8	12	76.4	16.8
4 ^c	MeOH/dioxane (1/1)	0.22	10	8	3	74.2	15.8
5	MeOH/dioxane (1/1)	0.33	10	8	16.5	71.9	19.2
6	MeOH/dioxane (1/1)	0.11	10	8	25	79.7	11.9
7	dioxane	0.22	10	8	23.5	85.0	11.2
8	dioxane	0.11	10	8	20.5	87.3	8.4

^a Water (4%) was also added. ^b HPLC area %. ^c H₂ pressure was 2 kg/cm³.

Table 2. Preparation of **6**

entry	X ^a	base (equiv)	solvent (M)	temp (°C)	time (h)	yield ^b (%)
1	Br	<i>i</i> -Pr ₂ EtN (5.6)	DMSO (0.1)	120	20	38
2	Br	<i>i</i> -Pr ₂ EtN (2.0)	DMSO (0.1)	100	20	51
3	Cl	<i>i</i> -Pr ₂ EtN (2.0)	DMSO (0.1)	100	20	42
4	Br	NaHCO ₃ (2.0)	DMSO (0.1)	100	20	25
5	Br	NaHCO ₃ (2.0)	EtOH (0.1)	78	26	47
6	Br	NaHCO ₃ (2.0)	<i>n</i> -BuOH (0.76)	100	19	72
7	Cl	NaHCO ₃ (2.0)	<i>n</i> -BuOH (0.76)	100	26	73

^a 2-X-pyrimidine. ^b The yield is not corrected for purity.

substituent to **5**, using the expensive 2-bromopyrimidine. We varied the base, solvent, halogenated pyrimidine, and temperature, and ran the reactions in Nautilus, an automated synthesizer. The information of Table 2 summarizes the conditions for some of the reactions investigated. Entry 1 lists the conditions used in the original synthesis, which include an excess of *i*-Pr₂EtN and a temperature of 120 °C. We discovered that only two equivalents of *i*-Pr₂EtN and a temperature of 100 °C were necessary (entry 2). However, when 2-bromopyrimidine was replaced with the inexpensive reagent, 2-chloropyrimidine, with all other conditions of entry 2 remaining the same, the yield decreased (entry 3). Replacing *i*-Pr₂EtN with NaHCO₃, and DMSO with EtOH, which necessitated a decrease in the reaction temperature, decreased the formation of byproduct, but the reaction then proceeded more slowly (compare entries 2 and 5). To improve the reaction speed, *n*-BuOH, which has the higher boiling point, replaced EtOH. Starting with either 2-bromopyrimidine or the inexpensive 2-chloropyrimidine now provided **6** in more than 70% yield,¹³ although the methyl group that protected the carboxyl was partially replaced with an *n*-Bu group (entries 6 and 7). We isolated **6** and the *n*-Bu ester derivative by the addition of MeOH, poured the mixture into water and filtration. Their ester moieties were removed by hydrolysis to give **7** in 80% yield (two steps) at 95.8% purity.

For the subsequent amidation of **7**, which gives **9**, the following changes were made. 1) Chromatographic purification was eliminated. Instead the product mixture was poured into water and solid **9** was isolated by filtration. 2) To decrease the reaction time, BOP¹⁷ (7 h at rt) replaced EDC (12 h at rt). 3) The reaction temperature was kept below 8 °C at all times to avoid coprecipitating a sticky yellow material with **9**. The yield of **9** was 96% and its assay purity was 94.4%.

If the *t*-Bu group could be removed in an acidic environment, the pyrimidine moiety of **9** would be in its acid salt, which is required for the final hydrogenation reaction. Therefore, we designed a procedure that combined these two reactions so that one purification step could be eliminated. During the original synthesis, acid (TFA) hydrolysis was used to remove the *t*-Bu group of **9**. To further decrease the cost of the synthesis, we replaced TFA with HCl, which is inexpensive, and removed the *t*-Bu group in 0.5 M HCl at 60 °C. (Not only did this procedure remove the *t*-Bu group, but it also left the pyrimidine moiety of the product as the HCl salt, which is required for the hydrogenation.) After cooling the mixture, we added Pd/C and, under an H₂ atmosphere, hydrogenated the product of the preceding step. Thus, a one-pot, one-step procedure consisting

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of the hydrolysis of the *t*-Bu group and the hydrogenation was achieved. An unidentified byproduct, with a molecular weight that is 16 Da greater than that of **1**, accumulated to a level of about 10%. After several trials, we found that production of this byproduct could be suppressed if, before adding Pd/C, the pH of the solution was adjusted to 1.7.

Our final task was to purify **1**. No crystallization method was known for **1**. We first surveyed many crystallization conditions. A chromatographically purified sample of **1** crystallized from an *i*-PrOH-water mixture. We next tried to crystallize **1** directly from the reaction mixture. First Pd/C was removed by filtration, then after adjusting the pH of the solution to 5, crystals of **1** were obtained in 56% yield¹³ with 87 area % by HPLC. Further investigation indicated that the maximum yield of **1**, achievable by crystallization, was obtained when the pH of the filtrate was pH 7. However, as the pH of the filtrate was increased, sometimes a gummy form of **1** precipitated. This problem was prevented by first adjusting the pH of the filtrate to 2.8, and then adding MeOH before neutralizing the solution. Crystals of **1**, obtained by this method, were isolated in 84% yield and were 96.5% pure. The value, 96.5%, was calculated using the purity of a standard (95.7%), the relative area of the HPLC peak found for **1** (98.3%), and the relative amount of residual water (2.6%) associated with the crystal. If crystalline **1** is the monohydrate form of the compound, then, theoretically, water accounts for 3.1% of the weight, a number that is very similar to 2.6%, and, in that case, the assay purity of **1** is 99%. The optical purity of **1** was >99%, no racemization was apparent.

Conclusions

A scalable synthesis of **1**, which is an antagonist for both integrins $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$, has been developed. We first developed a piperidine ring-closure route that, unlike the one used in the original procedure, does not use the expensive reagent **2**. Instead, we used the inexpensive reagent **12**. Many different efforts were made to decrease the number and types of steps required to synthesize **1** from **12** and some of these efforts resulted in the optimized route. Incorporating the direct reductive amination of **15** decreased the number of steps required to synthesize **5** from **15** by three, because steps d, e of Scheme 3 and steps c, d, e of Scheme 1 are no longer used. This reductive amination also did not use hazardous azides. To prepare **6**, the less expensive 2-chloropyrimidine could replace the expensive 2-bromopyrimidine if the base and the solvent were also changed. We discovered a crystallization condition that allowed us to isolate **1** directly from the reaction mixture in 84% yield and at 96.5% purity. By following our optimized procedure, **1** can be synthesized in eight steps, which are two steps less than the original synthesis. The overall yield of **1** is 45%, which is a 35% improvement compared with the yield obtained from the original synthesis. The new protocol does not involve chromatographic purifications, but instead relies only simple procedures, i.e. precipitations or crystallizations, that are more suited to the large-scale processes necessary for drug synthesis. Further, **1** is now available with a 98% cost saving relative to the original protocol. The overall yield (55%) and the purity (96.4%) of **1** synthesized from 50 g of **12** are very

similar to those found when 0.95 kg of **12** was used. Samples of compound **1**, which have been synthesized using this new route have already been used for preclinical and toxicological studies.

Experimental Section

¹H and ¹³C NMR spectra were recorded on JNM-LA400 spectrometers with chemical shifts reported in ppm with internal tetramethylsilane as a standard. Electron ionization (EI) mass spectra were recorded on a Hitachi M-80B instrument. Fast-atom bombardment (FAB) mass spectra were recorded on a JEOL JMS-700 instrument. Thermospray electrospray ionization (ESI) mass spectra were recorded on a Hewlett-Packard 5989A instrument. High-resolution mass spectra (HRMS) were recorded under FAB conditions. Optical rotation was obtained on JASCO DIP-370 polarimeter. Melting point was determined on a Yanaco micro melting point apparatus. 3-Hydroxy-4-nitrobenzoic acid (**12**) was purchased from Acros Organics. Diaminopropionate **8** was purchased from Peptide Institute, Inc. Elemental analyses were performed by the Toray Research Center and were within $\pm 0.5\%$ of calculated values. All assay yields were obtained by HPLC, using pure and characterized standards. HPLC purities were determined with Inertsil ODS-2 reversed phase column (4.6 mm \times 250 mm).

Methyl 3-methoxy-4-nitrobenzoate (13). DMF (9.5 L) and K₂CO₃ (1.44 kg, 10.5 mol) were added to 3-hydroxy-4-nitrobenzoic acid (**12**) (0.95 kg, 98% purity, 5.08 mol). To this suspension, was added methyl iodide (2.20 kg, 15.5 mol). The reaction mixture was stirred for 22 h at a temperature range of 25–30 °C. The mixture was poured slowly into cooled water (17 L), while maintaining the temperature between 13 and 25 °C. Resulting solids were collected, rinsed with water (6.8 L), and dried under reduced pressure for 16 h at room temperature to give **13** (1.04 kg, 95.3% yield, HPLC assay 98.3% (area)) as a pale-yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 3.97 (3H, s, OMe), 4.02 (3H, s, OMe), 7.70 (1H, dd, *J* = 1.6, 8.3 Hz, C₆H₃), 7.76 (1H, d, *J* = 1.6 Hz, C₆H₃), 7.84 (1H, d, *J* = 8.3 Hz, C₆H₃); EIMS *m/z* 211 M⁺. HPLC method: detector wavelength, 260 nm; flow rate, 0.8 mL/min; eluent, CH₃CN/H₂O = 50/50; temp, rt.

An analytical sample was purified with Inertsil ODS-2 column chromatography: Anal. Calcd for C₉H₉NO₅: C, 51.19; H, 4.30; N, 6.63. Found: C, 51.11; H, 4.28, N, 6.66.

Methyl 4-Amino-3-methoxybenzoate (11). To a solution of **13** (1.00 kg, 98.3% purity, 4.66 mol), 1,4-dioxane (5 L), and MeOH (5 L) was added 10% Pd/C (50 g) in water (1.0 L), and the mixture was hydrogenated under balloon pressure of hydrogen for 12 h at a temperature range of 35–40 °C. The mixture was filtered through Celite, and solids were washed with 1,4-dioxane/MeOH (1:1) (2 L). The combined filtrate and washings were concentrated to a final volume that was approximately 2 L. To the solution was added water (6.7 L), and resulting suspension was cooled to 12 °C for 1 h. The solids were collected by filtration, washed with water (5.0 L), and dried under reduced pressure at 35 °C for 18 h to provide **11** (811 g, 96.4% yield, HPLC assay 100.3% (area)) as a pale-yellow solid: ¹H NMR (400 MHz, CDCl₃) δ 3.86 (3H, s, OMe), 3.90 (3H, s, OMe), 6.66 (1H, d, C₆H₃), 7.46 (1H, d, C₆H₃),

7.55 (1H, dd, C₆H₃); EIMS *m/z* 181 M⁺. HPLC method: detector wavelength, 260 nm; flow rate, 1.0 mL/min; eluent, CH₃CN/H₂O = 50/50; temp, rt.

An analytical sample was purified with Inertsil ODS-2 column chromatography: Anal. Calcd for C₉H₁₁NO₃: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.66; H, 6.12, N, 7.73.

1,5-Dichloropentan-3-one (10). 3-Chloropropionyl chloride (1.40 kg, 11.0 mol) was dissolved in dichloromethane (1.7 L), and the solution was cooled to -30 °C. To this solution was added AlCl₃ (1.87 kg, 14.3 mol) slowly at a rate that kept the internal temperature of the mixture below 0 °C. The mixture was stirred at a temperature range of 10–20 °C under bubbling ethylene gas for 6 h. The reaction mixture was poured into a precooled (4 °C) mixture of water (5.8 L), conc. HCl (0.47 L), and dichloromethane (1.4 L), while the internal temperature of the mixture was maintained below 13 °C. Organic layer was separated, and aqueous layer was washed with dichloromethane (1.4 L) twice. Combined organic layer was washed with 1.6 L of water once and 3.0 L of water twice, dried over anhydrous MgSO₄ (310 g), and concentrated to give **10** (1.74 kg) as a black syrup: ¹H NMR (400 MHz, CDCl₃) δ 2.94 (4H, t, *J* = 6.5 Hz, COCH₂CH₂Cl), 3.76 (4H, t, *J* = 6.5 Hz, COCH₂-CH₂Cl).

Methyl 3-Methoxy-4-(4-oxopiperidin-1-yl)benzoate (15). To **11** (804 g, 100% purity, 4.45 mol) was added MeOH (8.0 L), and the suspension was warmed to 60 °C. To the resulting solution was added **10** (1.44 kg, 9.28 mol) and stirred for 4 h at a temperature range of 60–67 °C. The mixture was poured into water (16 L), and the resulting tar was filtrated with Celite. The filtrate was concentrated until the total volume of the residue was approximately 8.5 L. To the resulting water solution was added activated carbon (80 g) and stirred for 30 min at 50 °C. Insolubles were removed by filtration and washed with water (2.0 L). The combined filtrate and washings were adjusted to pH of 6.8–7.2 with NaHCO₃ (1.14 kg, 13.6 mol). Resulting solids were collected by filtration, washed with water (3.2 L), and dried under reduced pressure for 44 h at 40 °C to give **15** (1.01 kg, 83.8% yield, HPLC assay 97.2% (area)) as a colorless solid: ¹H NMR (400 MHz, CDCl₃) δ 2.64 (4H, t, *J* = 5.8 Hz, piperidine), 3.46 (4H, t, *J* = 5.8 Hz, piperidine), 3.90 (3H, s, OMe), 3.96 (3H, s, OMe), 6.94 (1H, d, *J* = 8.2 Hz, C₆H₃), 7.56 (1H, d, *J* = 1.7 Hz, C₆H₃), 7.65 (1H, dd, *J* = 1.7, 8.2 Hz, C₆H₃); EIMS *m/z* 263 M⁺. HPLC method: detector wavelength, 300 nm; flow rate, 1.0 mL/min; eluent, CH₃CN/H₂O = 50/50; temp, rt.

An analytical sample was purified with Inertsil ODS-2 column chromatography: Anal. Calcd for C₁₄H₁₇NO₄: C, 63.87; H, 6.51; N, 5.32. Found: C, 64.00; H, 6.63, N, 5.35.

Methyl 4-(4-Aminopiperidin-1-yl)-3-methoxybenzoate (5). To a solution of **15** (311 g, 97.2% purity, 1.15 mol) and 1,4-dioxane (10.5 L) was added 10% Pd/C (35.0 g) in water (0.4 L) and 28% aqueous ammonia (2.8 L). The mixture was hydrogenated under balloon pressure of hydrogen for 12 h at a temperature range of 21–28 °C. The mixture was filtered through Celite, and solids were washed with 1,4-dioxane/MeOH (1:1) (1.0 L). The combined filtrate and washings were concentrated until the remaining volume was approximately 2.8 L, water was added (2.8 L), and this was concentrated again

until the remaining volume was approximately 3.5 L. The mixture was adjusted to pH of 6.9–7.1 with 2 M HCl (520 mL). Insoluble byproduct **17** was washed with AcOn-Bu (1.8 L) three times, maintaining the pH of the aqueous layer. To the aqueous layer (4.5 L) was added NaCl (899 g) and the pH adjusted to 9.8 with 2 M aqueous K₂CO₃ (980 mL). The mixture was extracted with AcOEt (6.3 L, 2.6 L × 2) three times, while the pH of the aqueous layer was maintained upper 9.8 with 2 M aqueous K₂CO₃. Combined organic layer was concentrated and dried under reduced pressure for 16 h at 50 °C to give **5** (276 g, 90.4% yield, HPLC assay 99.4% (area)) as a colorless solid: ¹H NMR (400 MHz, CDCl₃) δ 1.58 (2H, dddd, *J* = 3.9, 10.5, 11.6, 12.7 Hz, piperidine), 1.95 (2H, br d, *J* = 12.7 Hz, piperidine), 2.70 (2H, ddd, *J* = 2.4, 11.6, 12.4 Hz, piperidine), 2.82 (1H, tt, *J* = 4.3, 10.5 Hz, piperidine), 3.57 (2H, br d, *J* = 12.4 Hz, piperidine), 3.88 (3H, s, OMe), 3.92 (3H, s, OMe), 6.91 (1H, d, *J* = 8.3 Hz, C₆H₃), 7.50 (1H, d, *J* = 1.8 Hz, C₆H₃), 7.62 (1H, dd, *J* = 1.83, 8.3 Hz, C₆H₃). HPLC method: detector wavelength, 300 nm; flow rate, 1.0 mL/min; eluent, CH₃CN/2% aqueous NH₄OAc = 45/55; temp, rt.

Methyl 3-Methoxy-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (6). To a solution of **5** (270 g, 99.4% purity, 1.01 mol) in *n*-BuOH (1.35 L), 2-chloropyrimidine (117 g, 1.02 mol) and NaHCO₃ (171 g, 2.04 mol) were added. The reaction mixture was heated to 100–110 °C for 27 h. The cooled (50 °C) mixture was added MeOH (2.7 L) and poured into water (13.5 L). Resulting suspension was stirred for 2.5 h under cooling with an ice bath. Solids were collected by filtration, washed with water (1.0 L), and dried with P₂O₅ for 2 days to give a mixture of **6** and *n*-butyl 4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoate (297 g, 71.1% yield, HPLC assay of **6**) 82.7%, HPLC area % of Bu ester: 8.4%) as a pale fellow solid: HPLC method: detector wavelength, 300 nm; flow rate, 1.0 mL/min; eluent, CH₃CN/2% aqueous NH₄OAc = 50/50; temp, rt.

An analytical sample was purified with Inertsil ODS-2 column chromatography: ¹H NMR of **6** (400 MHz, CDCl₃) δ 1.75 (2H, dddd, *J* = 3.7, 10.7, 11.0, 12.9 Hz, piperidine), 2.20 (2H, br d, *J* = 12.9 Hz, piperidine), 2.86 (2H, ddd, *J* = 2.3, 10.7, 12.8 Hz, piperidine), 3.58 (2H, br d, *J* = 12.8 Hz, piperidine), 3.89 (3H, s, CO₂Me), 3.93 (3H, s, C₆H₃OMe), 3.96–4.07 (1H, m, piperidine), 6.54 (1H, t, *J* = 4.9 Hz, pyrimidine), 6.94 (1H, d, *J* = 8.3 Hz, C₆H₃CO), 7.51 (1H, d, *J* = 1.9 Hz, C₆H₃CO), 7.64 (1H, dd, *J* = 1.9, 8.3 Hz, C₆H₃CO), 8.29 (2H, d, *J* = 4.9 Hz, pyrimidine); ESIMS of **6** *m/z* 343 (M + H)⁺; Anal. Calcd for C₁₈H₂₂N₄O₃: C, 63.14; H, 6.48; N, 16.36. Found: C, 63.34; H, 6.53, N, 16.42.

3-Methoxy-4-{4-(pyrimidin-2-ylamino)piperidin-1-yl}benzoic Acid (7). To a solution of a mixture of **6** and the butyl ester derivative (290 g, 82.7% purity, 0.701 mol) in MeOH (1.45 L), was added 1.2 M NaOH (1.45 L). The reaction mixture was stirred for 2 h at a temperature range of 50–60 °C. The mixture was concentrated until the remaining volume was approximately 1.45 L, water (580 mL) was added, and the mixture was concentrated again until the remaining volume was approximately 1.45 L. The pH of the residue was adjusted to 4.5 with 2 M HCl (855 mL), while the temperature was maintained between 7 and 10 °C. To the mixture was added water (595 mL). Resulting solids were collected by filtration,

washed with water (600 mL), and dried under reduced pressure for 16 h at the room temperature to give **7** (272 g, 113% yield, HPLC assay 95.8% (area)) as a colorless solid: $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 1.64 (2H, dddd, $J = 3.5, 10.5, 11.6, 12.7$ Hz, piperidine), 1.93 (2H, br d, $J = 12.7$ Hz, piperidine), 2.70 (2H, br dd, $J = 11.6, 12.2$ Hz, piperidine), 3.50 (2H, br d, $J = 12.2$ Hz, piperidine), 3.83 (3H, s, C₆H₃OMe), 3.83–3.90 (1H, m, piperidine), 6.55 (1H, t, $J = 4.7$ Hz, pyrimidine), 6.93 (1H, d, $J = 8.3$ Hz, C₆H₃CO), 7.41 (1H, d, $J = 1.8$ Hz, C₆H₃CO), 7.49 (1H, dd, $J = 1.8, 8.3$ Hz, C₆H₃CO), 8.27 (2H, d, $J = 4.7$ Hz, pyrimidine); ESIMS m/z 329 (M + H)⁺. HPLC method: detector wavelength, 305 nm; flow rate, 1.0 mL/min; eluent, CH₃CN/2% aqueous NH₄OAc = 50/50; temp, rt.

An analytical sample was washed with AcOEt, CH₃CN and H₂O: Anal. Calcd for C₁₇H₂₀N₄O₃: C, 62.18; H, 6.14; N, 17.06. Found: C, 62.47; H, 6.25, N, 17.09.

tert-Butyl (2S)-Benzenesulfonylamino-3-[3-methoxy-4-(pyrimidin-2-ylamino)piperidin-1-yl]benzoylamino]propionate (9). The carboxylic acid **7** (112 g, 95.8% purity, 0.326 mol) and *N*-methylmorpholine (41.3 g, 0.480 mol) were suspended in DMF (1.12 L) and cooled to 4 °C; (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent) (165 g, 0.374 mol) was added slowly, while the temperature was maintained at 4 °C. After 30 min, *tert*-butyl (2S)-*N*-benzenesulfonyl-2,3-diaminopropionate **8** (102 g, 0.341 mol) was added to the mixture at a rate that kept the internal temperature of the mixture below 7 °C. The mixture was stirred an additional 23 h at a temperature range of 3–8 °C and then was poured into water (5.6 L) over a 20-min period. The reaction flask was washed with acetone (70 mL), and the washing was also poured into the mixture. Resulting solids were collected by filtration and washed with water (700 mL), and 5% aqueous NaHCO₃ (10 L) was added. After stirring for 30 min, the solids were collected by filtration and washed with water (1.15 L) until the pH of the filtrate was below 6.5. The solid was dried under reduced pressure for 14 h at room temperature, for 22 h at 40 °C, and for 23 h with P₂O₅ to give **9** (202 g, 96.0% yield, HPLC assay 94.4% (area)) as a colorless solid: $^1\text{H NMR}$ (400 MHz, CDCl₃) δ 1.29 (9H, s, *t*-Bu), 1.76 (2H, br dddd, $J = 4.1, 10.4, 11.5, 12.9$ Hz, piperidine), 2.20 (2H, br d, $J = 12.9$ Hz, piperidine), 2.85 (2H, br dd, $J = 11.5, 11.5$ Hz, piperidine), 3.47–3.59 (3H, m, piperidine and CONHCH₂CH), 3.88–4.06 (3H, m, piperidine and CONHCH₂CH), 3.95 (3H, s, C₆H₃OMe), 6.54 (1H, t, $J = 4.8$ Hz, pyrimidine), 6.95 (1H, d, $J = 8.2$ Hz, C₆H₃CO), 7.33 (1H, dd, $J = 2.0, 8.2$ Hz, C₆H₃CO), 7.41 (1H, d, $J = 2.0$ Hz, C₆H₃CO), 7.50 (2H, br dd, $J = 7.1, 7.4$ Hz, C₆H₅), 7.58 (1H, br t, $J = 7.4$ Hz, C₆H₅), 7.86 (2H, br d, $J = 7.1$ Hz, C₆H₅), 8.29 (2H, d, $J = 4.8$ Hz, pyrimidine); FABMS m/z 611 (M + H)⁺. HPLC method: detector wavelength, 305 nm; flow rate, 1.0 mL/min; eluent, CH₃CN/phosphate buffer Na (pH) = 40/60; temp, 40 °C.

An analytical sample was purified with Inertsil ODS-2 column chromatography: Anal. Calcd for C₃₀H₄₀N₆O₇S (as monohydrate): C, 57.31; H, 6.41; N, 13.37. Found: C, 57.40; H, 6.47, N, 13.41.

(2S)-Benzenesulfonylamino-3-[3-methoxy-4-(1,4,5,6-tetrahydropyrimidin-2-ylamino)piperidin-1-yl]benzoylamino] propionic Acid (1). A solution of **9** (200 g, 94.4% purity, 0.309 mol) in 0.5 M HCl (4.0 L) was warmed at a temperature range of 60–61 °C and stirred for 5.5 h. The mixture was cooled to 35 °C and water was added (4.0 L). The pH of the mixture was adjusted to 1.6–1.7 with 5 M aqueous NaOH (226 mL). To the mixture was added 10% Pd/C (10.0 g) in water (400 mL), and this was hydrogenated under balloon pressure of hydrogen for 7.5 h at a temperature range of 30–36 °C. Insolubles were filtrated and washed with 0.1 M HCl (4.7 L). The filtrate and washing were combined, and the solution was adjusted to pH of 2.8–2.9 by adding 5 M NaOH (148 mL). To the mixture was added MeOH (2.0 L) and pH adjusted to 5.0–5.2 with 5 M aqueous NaOH (96 mL). The pH of the mixture was further adjusted to pH of 7.4–7.6 carefully with 1 M aqueous NaOH (22.5 mL). The resulting solution was seeded with an authentic sample and stirred slowly for 20 h. The solvents (about 1.6 L) were removed via evaporation. Solids were collected by filtration and dried under reduced pressure for 18 h at 35 °C to give **1** (149 g, 83.5% yield, HPLC assay 96.5% (area), >99% ee) as a colorless solid: $^1\text{H NMR}$ (400 MHz, DMSO- d_6) δ 1.39–1.43 (2H, m, piperidine), 1.73–1.80 (4H, m, piperidine and tetrahydropyrimidine), 2.39–2.47 (2H, m, piperidine), 3.08–3.15 (2H, m, CONHCH₂CH and piperidine), 3.19 (4H, m, tetrahydropyrimidine), 3.28 (2H, m, piperidine), 3.48 (1H, m, CONHCH₂CH), 3.60 (1H, m, CONHCH₂CH), 3.68 (3H, s, C₆H₃OMe), 6.77 (1H, d, $J = 8.7$ Hz, C₆H₃CO), 7.26 (1H, d, $J = 8.7$ Hz, C₆H₃CO), 7.28 (1H, s, C₆H₃CO), 7.53–7.57 (2H, m, C₆H₅), 7.61 (1H, m, C₆H₅), 7.83 (1H, d, $J = 6.8$ Hz, C₆H₅), 7.84 (1H, d, $J = 6.8$ Hz, C₆H₅), 8.31 (1H, br t, $J = 5.0$ Hz, CONHCH₂CH), 8.71 (1H, br s, NH), 9.42 (1H, br d, $J = 7.6$ Hz, NH); $^{13}\text{C NMR}$ (DMSO- d_6) δ 19.8, 31.8, 32.1, 37.8, 42.6, 47.3, 48.9, 49.0, 55.3, 55.4, 110.5, 117.2, 119.6, 126.6, 128.3, 129.0, 132.3, 140.8, 143.9, 151.3, 152.0, 165.4, 173.5; FABMS m/z 559 (M + H)⁺; FAB-HMS (M + H)⁺ calcd for C₂₆H₃₄N₆O₆S: 559.2339, found: 559.2343. HPLC methods: (A) Nonchiral HPLC conditions: detector wavelength, 305 nm; flow rate, 1.0 mL/min; eluent, CH₃CN/10 mM sodium phosphate buffer = 40/60 or 20/80 (for hydrogenation); temp, 40 °C. (B) Chiral HPLC conditions: column, sumichiral OA-3200; detector wavelength, 305 nm; flow rate, 1.0 mL/min; eluent, MeOH/0.5 mM aqueous NH₄OAc; temp, rt.

An analytical sample was prepared by recrystallization from EtOH/H₂O = 2/1: mp 162–164 °C; Anal. Calcd for C₂₆H₃₄N₆O₆S 1.68 H₂O: C, 53.03; H, 6.39; N, 14.27. Found: C, 52.60; H, 5.95; N, 14.12; $[\alpha]_D^{25} +92^\circ$ (*c* 0.31, CHCl₃/MeOH = 1:1).

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